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Graft versus host disease (GVHD) is a significant cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation. In vivo quantitative T-cell depletion using CAMPATH-1H (anti-CD52) has been explored in an effort to prevent acute GVHD. Recently, a regimen of total lymphoid irradiation (TLI) and anti-thymocyte globulin (ATG) has been shown to polarize T-cells towards an inhibitory phenotype potentially reducing the risk for GVHD. However, these strategies may lead to impaired post-transplant immune reconstitution, increased risk of tumor relapse and opportunistic infection. We compared the immune recovery of 20 patients undergoing reduced intensity conditioning with low dose CAMPATH and an initial cohort of 5 patients treated with TLI/ATG. Conditioning with CAMPATH resulted in a significant depletion of CD4 and CD8 T-cells in the early post-transplant period and persistence of CD4 T-cell depletion for 6 months. Following TLI/ATG, there was a persistent depletion of CD4 T-cells with no significant decrease in CD8 T-cells. CAMPATH was associated with a decrease in CD45RO+ memory T-cells in the early post-transplant period (27.2 to 5.7%, $p = 0.03$). T-cell recovery in early post-transplant following TLI/ATG was associated with a rise in the relative percentages of naive T-cells (CD45RA+) (39 to 61.3%; $p = 0.04$), central memory (CD45RO + CD62L + CCR7+) (CM) (12 to 32.8%; $p = 0.05$), and a significant change in the central memory:effector memory (CD45RO + CD62L-CCR7-) (EM) ratio (0.2 to 1.0). The mean percentage of regulatory T-cells (CD4 + CD25 + FoxP3+) rose in the early post-transplant period following both regimens ($p < 0.03$). Functional analyses demonstrated that the T-cell proliferative response to Phytohemagglutinin (PHA) was profoundly depressed following CAMPATH with mean SI decreasing from 34 pre-transplant to 1.4 Day 30. Treatment with TLI/ATG resulted in no significant change in response to PHA. Assessment of T-cell polarization after stimulation with PMA/ionomycin, recipient derived dendritic cells (DCs) or third party DCs demonstrated a rise of CD8 + T-cells expressing IL-4 and IL-10 consistent with a suppressor phenotype. In summary, both CAMPATH and TLI/ATG result in CD4 + T-cell depletion, but TLI/ATG resulted in persistence of memory cells, relative preservation of CM as compared to EM and intact response to mitogens. TLI/ATG therapy was associated with a more modest level of functional T-cell depletion characterized by Tc2 polarization.

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CD4 + CD25HIGHFOXP3 + REGULATORY T CELLS ARE INCREASED AND FUNCTIONALLY ACTIVE AFTER ANTITHYMOCYTE GLOBULIN INFUSION AND ALLOGENEIC STEM CELL TRANSPLANTATION IN HUMANS – A NOVEL IN VIVO MECHANISM OF ACTION

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CD4 + CD25highFoxP3+ regulatory T cells (Tregs) play a central role in immunologic homeostasis and are able to induce tolerance after allogeneic hematopoietic stem cell transplantation (alloPBSCT). Polyclonal rabbit-anti-human antithymocyte globulins (rATG) are widely used to prevent and treat Graft-versus-Host-Disease (GvHD) after alloPBSCT. Their mechanism of action has been thought to be mainly mediated by CD4+ effector T cell depletion and complement-dependent lysis. Interestingly, recent in vitro data have suggested that rATGs might increase the ratio or number of regulatory T cells. But so far, it is not known whether such effects exist in humans in vivo.

Here, we present the data of a prospective pilot study in which we analyzed the influence of rATGs on the reconstitution of peripheral CD4+/CD25high/FoxP3 + Tregs after alloPBSCT.

Ten patients were divided into two groups depending on the requirements of the respective alloPBSCT protocol: those who received one type of rATG during conditioning therapy from day -3 to -1 (rATG; n = 6) and those who were conditioned without rATG administration (non-rATG; n = 4). Patients of the rATG group received a total dose of either rATG-Fresenius 30 mg/kgBW (n = 3) or rATG-Genzyme 6 mg/kgBW (n = 3) respectively. All patients received peripheral hematopoietic stem cells from HLA-matched donors and the grafts contained comparable median numbers of CD3+ and CD34+ in both patient groups (table 1). GvHD prophylaxis consisted of Mycophenolate Mofetil and Cyclosporine A in all patients and antiviral prophylaxis was with acyclovir. Peripheral blood samples were taken before conditioning and after alloPBSCT at distinct time points (day +30, +60, +90 and +150) and immune cells were analyzed by flow cytometry. An in vitro suppression assay of d + 150 Tregs from cryopreserved samples was done with CD4 + CD25+ selected Tregs and CD4 + CD25- selected CSFE labelled autologous responder T cells.

In summary, we found a more than 3-fold stable increase of relative Treg numbers in rATG treated patients after alloPBSCT compared to the control. Day + 150 Tregs suppressed the responder cell proliferation effectively in the in vitro assay.

Taken together, our results rise first evidence that rATG treatment leads to increased, functional active CD4+/CD25high/FoxP3 + Tregs in humans after allogeneic stem cell transplantation which supports the thesis that rATG exhibit their immunomodulatory activity by additional mechanisms beyond simple cell depletion.

Table 1. Patient characteristics and transplantation parameters

Baseline parameters	rATG	non-rATG	
Patients	6	4	
Age (median)	37 - 68 (61)	48 - 67 (62)	
Diagnosis	MM, MCL, CLL, ALL	AML (n = 3), OMF	
Conditioning regimen			
RIC	5	3	
Standard	1	1	
Transplanted cell numbers x 106/kg BW recipient, (median)			
CD34 + PBSC	1.85 - 11.0 (7.6)	4.91 - 9.47 (7.3)	p 0.47
CD3 + T cells	150 - 480 (219)	120 - 540 (175)	p 0.38

MRD: matched related donor; MUD: matched unrelated donor; RIC: Reduced Intensity Conditioning (Treosulfan / Fludarabin or Cyclophospamide/Fludarabin or 2 Gy-Total-Body-Irradiation/ Fludarabin); Standard conditioning (Cyclophosphamide/12 Gy-Total-Body-Irradiation). BW: body weight; AML: acute myeloid leukaemia; OMF: osteomyelofibrosis; CLL: chronic lymphocytic leukaemia; ALL: acute lymphoblastic leukaemia; MCL: mantle cell lymphoma; MM: multiple myeloma. PBSC: peripheral blood stem cells. Cellular subpopulations of the transplanted grafts were calculated to the respective recipients body weight. Statistical analysis by SPSS.

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ADOPTIVE TRANSFER OF EBV SPECIFIC T-CELLS FOR TREATMENT OF PRIMARY AND RITUXAN RESISTANT EBV LYMPHOMAS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTS (HSCT): CLINICAL, VIRAL AND IMMUNOLOGIC CORELATES

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EBV lymphomas (EBV-L) arising post HSCT that persist or recur following Rituxan therapy are often fatal. We analyzed treatment with EBV-specific T-cells (EBV-CTL) in 22 patients (pts) who developed EBV-L following HLA-matched (N = 8) or non-identical N = 11 T cell depleted (N = 17) or unmodified (N = 3) HSCT or cord blood grafts (N = 3). All pts had clinical and radiologic evidence of rapidly growing tumors of Waldeyer's ring and/or intestines, liver, lung or CNS and rising blood levels of EBV DNA. Biopsies showed B cell, EBV^+ lymphoma in 20 pts that were monoclonal (12/12 tested) and usually of donor origin (11/12 tested). Of 22 pts, 14 had failed (N = 10) or recurred (N = 4) following Rituxan treatment. EBV-CTL were grown from donor T-cells sensitized with autologous EBVBLCL transformed by EBV strain B95.8 (B95.8 EBVBLCL) and tested for specificity, lack of alloreactivity and sterility. Treatment included 3 weekly infusions of EBV-CTL (10^6 T